Comparison of Three Management Strategies for Patients With Atypical Squamous Cells of Undetermined Significance: Baseline Results From a Randomized Trial

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For the ALTS Group

Background: **More than 2 million U.S. women receive an equivocal cervical cytologic diagnosis (atypical squamous cells of undetermined significance [ASCUS]) each year. Effective colposcopy triage strategies are needed to identify the minority of women who have clinically significant disease while avoiding excessive follow-up evaluation for others.** ***Methods:*** **The ASCUS/LSIL (i.e., low-grade squamous intraepithelial lesion) Triage Study (ALTS) is a multicenter, randomized trial comparing the sensitivity and specificity of the following three management strategies to detect cervical intraepithelial neoplasia grade 3 (CIN3): 1) immediate colposcopy (considered to be the reference standard), 2) triage to colposcopy based on human papillomavirus (HPV) results from Hybrid Capture 2™ (HC 2) and thin-layer cytology results, or 3) triage based on cytology results alone. This article summarizes the cross-sectional enrollment results for 3488 women with a referral diagnosis of ASCUS. All statistical tests are two-sided.** ***Results:*** **Among participants with ASCUS, the underlying prevalence of histologically confirmed CIN3 was 5.1%. Sensitivity to detect CIN3 or above by testing for cancer-associated HPV DNA was 96.3% (95% confidence interval [CI] = 91.6% to 98.8%), with 56.1% of women referred to colposcopy. Sensitivity of a single repeat cytology specimen with a triage threshold of HSIL or above was 44.1% (95% CI = 35.6% to 52.9%), with 6.9% referred. Sensitivity of a lower cytology triage threshold of ASCUS or above was 85.3% (95% CI = 78.2% to 90.8%), with 58.6% referred.** ***Conclusions:*** **HC 2 testing for cancer-associated HPV DNA is a viable option in the management of women with ASCUS. It has greater sensitivity to detect CIN3 or above and specificity comparable to a single additional cytologic test indicating ASCUS or above. [J Natl Cancer Inst 2001;93:293–9]**

Of the estimated 50 million Pap smears performed each year in the United States, more than 5% are reported as abnormal. There is general consensus by health care providers that cytologically diagnosed high-grade squamous intraepithelial lesions (HSILs) should be evaluated by colposcopy and biopsy. However, there is currently no consensus as to the appropriate management of the estimated 3 million women with low-grade squamous intraepithelial lesions (LSILs) or equivocal cytologic abnormalities (atypical squamous cells of undetermined significance [ASCUS]). Options include immediate colposcopy and directed biopsy as with cytologic HSILs, follow-up with repeat cytology every 4–6 months with colposcopy indicated only if an abnormality persists, or triage using DNA testing for cancer-associated human papillomavirus (HPV) types *(1).*

The ASCUS/LSIL Triage Study (ALTS) is a randomized, multicenter clinical trial of the management of women with low-grade and equivocal cervical cytology abnormalities. Sponsored by the National Cancer Institute *(2),* the study compares the sensitivity and specificity of immediate colposcopy, repeat cytology, and HPV testing for the timely detection of cervical intraepithelial neoplasia grade 3 (CIN3). The main study endpoint of histologically confirmed CIN3 was chosen because there is general consensus that this lesion is at high risk of progressing to invasive cancer and requires definitive treatment.

At the start of the trial, participants were randomly assigned to one of three management arms: 1) immediate colposcopy (all women go to colposcopy), 2) conservative management (colposcopy only if enrollment or any follow-up cytology is HSIL or above [HSIL+]), or 3) HPV triage (colposcopy only if the enrollment HPV test is positive or missing or any cytology is HSIL+). The HPV triage arm for women referred with a cytologic diagnosis of LSIL was closed early because an interim analysis showed that 83% of these women would be triaged to colposcopy based on a positive HPV result *(3).* While confirming the very high percentage of LSILs attributable to infection with cancer-associated HPV types, the results demonstrated limited utility of the HPV assay to direct management decisions for cytologic LSIL results because of the substantial majority of women referred to colposcopy with positive HPV tests. Enrollment into the remaining arms of the study closed as scheduled in December 1998, with a total of 5060 enrolled women referred with a recent diagnosis of either ASCUS (n = 3488) or LSIL (n = 1572).

This article summarizes the cross-sectional enrollment cytology and HPV DNA test results for women referred with a community diagnosis of ASCUS. Study participants are followed at 6-month intervals for a total of 2 years. At the conclusion of the trial, the sensitivity and cost-effectiveness of the various management strategies for detection of histologically confirmed CIN3 will be compared more definitively for all arms of the study.

Subjects and Methods

Enrollment Visit

The ALTS trial and characteristics of the enrollees are described more completely elsewhere *(2).* Enrollment took place from November 1996 through De-

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See "Appendix" section for affiliations of the ALTS Group.

See "Notes" following "References."

cember 1998 at four clinical centers: the University of Alabama (Birmingham, AL), Magee-Womens Hospital of the University of Pittsburgh Medical Center Health System (Pittsburgh, PA), the University of Oklahoma (Oklahoma City, OK), and the University of Washington (Seattle, WA). The study was approved by local institutional review boards. Women were enrolled an average of 2 months after the index referral smear was obtained. After eligibility was determined and written informed consent was obtained from the subjects, the participants were randomly assigned to one of the three management arms outlined earlier. Fig. 1 shows the overall study design, the number of participants enrolled with ASCUS, the randomization assignments, and the initial management.

Nurse–clinicians conducted the enrollment pelvic examination and collected two cervical specimens. The first cervical specimen was collected with a Papette™ broom (Wallach Surgical, Orange, CT) and was rinsed directly into a PreservCyt™ vial (Cytyc Corporation, Boxborough, MA). This specimen was used for both the preparation of ThinPrep™ (Cytyc Corporation) cytologic specimens and for HPV testing using Hybrid Capture 2™ (HC 2) (Digene Corporation, Gaithersburg, MD). The second cervical specimen, collected with a Dacron swab, was obtained for investigational HPV DNA typing; these results were not used in the trial. After the cervical specimens were collected, the cervix was rinsed twice with a 5% solution of acetic acid, and two Cervigrams™ (National Testing Laboratories, Fenton, MO) were taken. Finally, blood specimens were obtained for investigational immunologic studies.

Women randomly assigned to the immediate colposcopy arm proceeded immediately to colposcopy or were given an appointment to return for the procedure within 3 weeks if colposcopy could not be performed the same day. Women randomly assigned to the HPV triage arm were called back for colposcopy if the HPV test was positive or not done (missing) or if there was an ALTS clinical center enrollment cytology diagnosis of HSIL, a glandular abnormality, or cancer (these diagnoses as a group have been termed HSIL+). A missing HPV test result was most commonly due to insufficient (<4 mL) residual specimen in the PreservCyt vial (after preparing the ThinPrep) to perform the assay. Because it was considered to be an impractical triage strategy to recall women for repeat collection of a specimen for the HPV test alone, women in the HPV triage arm with no HPV test results were triaged to colposcopy. However, women with a clinical center enrollment cytology diagnosis of "unsatisfactory" in the HPV triage arm were recalled for repeat specimen collection, according to current practice (unless they were already triaged to colposcopy on the basis of a positive HPV test). In the conservative management arm, only women with a clinical center cytology diagnosis of HSIL or higher were referred to colposcopy. Unsatisfactory cytology in the conservative management arm also led to recall for repeat specimen collection. Clinicians very rarely (n = 6) referred patients to colposcopy on the basis of visualizing a lesion suspicious for cancer during the pelvic examination.

Community Referral Slide Review

All women had a community-read cytology result of ASCUS (termed "referral slide") as a prerequisite for study entry. These slides were requested from the community laboratories and were sent to the Pathology Quality Control Group (*see below*) for re-review.

Processing and Interpretation of Enrollment Specimens

Cytology slides. Liquid-based, ThinPrep cytology slides were prepared from PreservCyt vial specimens according to the manufacturer's standard protocol. Slides were screened at each clinical center by a cytotechnologist and evaluated by a cytopathologist trained to read ThinPreps according to routine practice. Cytologic results were recorded on a standardized data collection form with the use of the Bethesda System with subcategorical distinctions between HSIL-cervical intraepithelial neoplasia grade 2 (HSIL-CIN2) and HSIL-CIN3. After the clinical center evaluation, slides were sent to the Pathology Quality Control Group (*see below*) for rescreening and re-review.

HPV HC 2 test. Following the preparation of the ThinPrep, the PreservCyt vial was forwarded for HPV testing with the use of 4 mL of the residual specimen and the HC 2 assay to detect cancer-associated HPV types. If less than 4 mL remained, the specimen was considered to be unsatisfactory and the assay was not done.

The HC 2 assay includes a mixture of probes for the following cervical cancer-associated HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The U.S. Food and Drug Administration-approved threshold of 1 pg of HPV DNA/mL of test solution was used for a positive result *(4).* The low-risk probe set (types 6, 11, 42, 43, and 44) was not used. The HPV Quality Control Group monitored the performance of the HPV assay by using mock specimen controls with each run as well as by random retesting of a percentage of the clinical specimens.

Colposcopic Examination and Treatment of Histologically Confirmed Lesions

The colposcopic examination was performed according to routine practice except that a computer-assisted digital imaging system (Denvu™; DenVu, Tucson, AZ) was used by the colposcopist to capture images of the cervix and to record the biopsy sites selected. Biopsy specimens, obtained for any colposcopically suspected CIN, were placed in separate prelabeled vials containing 10% buffered formalin. Endocervical curettage was performed according to clinician judgment in cases where the entire transformation zone or extent of a lesion was not visualized adequately.

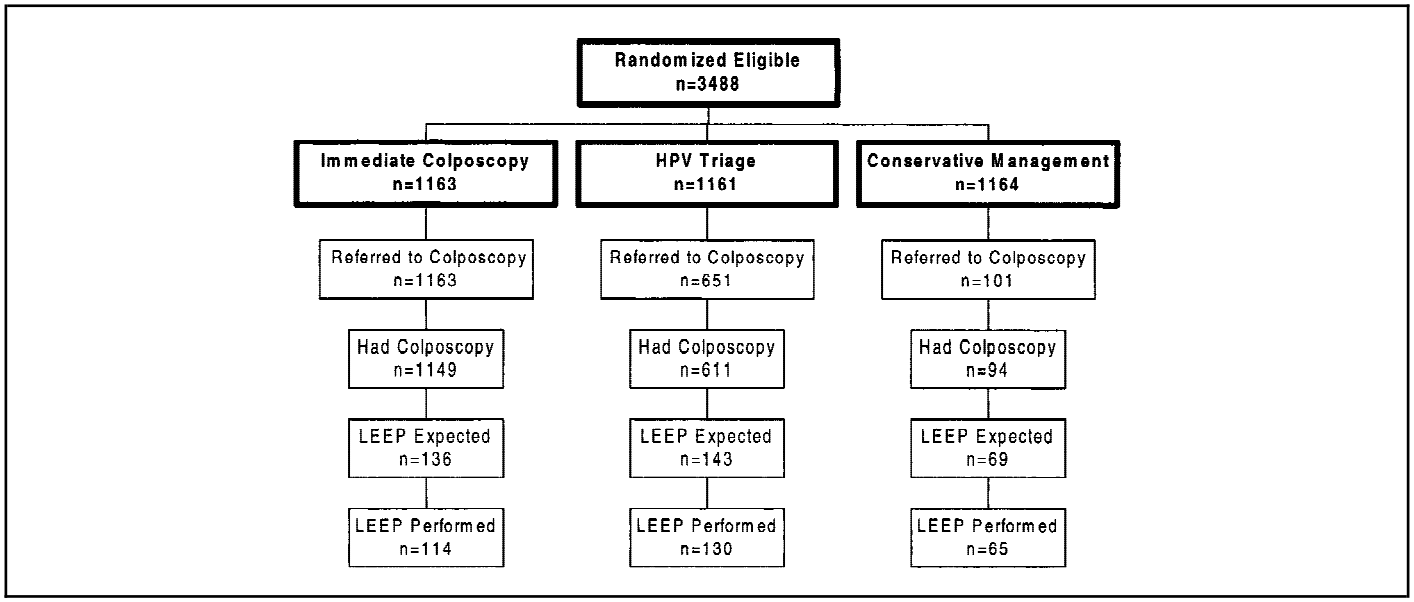

Fig. 1. Patients with atypical squamous cells of undetermined significance (ASCUS) in the ASCUS/Low-Grade Squamous Intraepithelial Lesion Triage Study (ALTS): enrollment, randomization, and initial management. HPV = human papillomavirus; LEEP = loop electrosurgical excision procedure.

Histologic interpretation of biopsy specimens was conducted at each center using a combination of the Bethesda System *(5)* and CIN *(6)* terminologies. After the interpretation by the center, all histology slides were sent to the Pathology Quality Control Group for re-evaluation.

As per standard practice, all histologically confirmed high-grade lesions diagnosed as CIN2 or above (CIN2+) by the clinical centers were treated by the loop electrosurgical excision procedure (LEEP) of the transformation zone. Histologically confirmed CIN1 was not treated and is being followed in a prospective cohort study.

If the colposcopically directed biopsy result was not consistent with a high-grade cytology or a high-grade colposcopy impression at the clinical center, a repeat colposcopy was performed according to clinician judgment to clarify diagnostic discrepancies. Such additional procedures, if performed within 1 year of enrollment as part of the continued work-up of a patient, are included in the enrollment database.

Pathology Quality Control Group Reviews

All referral slides, enrollment ThinPreps, and enrollment histology slides were sent to the Pathology Quality Control Group based at The Johns Hopkins Hospital, Baltimore, MD, for re-review and final case definition. For histology slides, the Pathology Quality Control Group review protocol included review by a quality control pathologist who was masked to the original diagnosis. Any case with a diagnosis of CIN2+, by either the Pathology Quality Control Group or the original clinical center, automatically went to a panel review composed of two of the four quality control pathologists unmasked to previous histology diagnoses. For all other histology cases, the first quality control review diagnosis was compared with the clinical center diagnosis; if concordant, that diagnosis served as the final diagnosis. In the event of disagreement between the clinical center and the first quality control reviewer, the case was sent to panel review. For all cases sent to panel review, that review constituted the final diagnosis.

Safety Notifications

In addition to providing expert interpretation for purposes of disease definition, the Pathology Quality Control Group review was also designed to provide a safety net for study participants. Clinical centers were notified by fax if there was concern for missed clinically significant disease. For referral smears, enrollment ThinPreps, and biopsy specimens, a Pathology Quality Control Group diagnosis of CIN3+ (which had been called less than CIN2 at the center) elicited a safety notification.

Enrollment cervigrams and digital colposcopic images also underwent external review for safety purposes. The threshold for safety notification for cervicography and digital colposcopic images was "suspect cancer."

Following a safety notification, the clinical center principal investigator reviewed each case to determine if re-evaluation of the patient was needed. The entire process—specimen collection, initial interpretation by the clinical center, transmittal to and review by the quality control group, communication of alerts to the centers, and additional patient evaluation as necessary—sometimes took many months to complete. Therefore, any colposcopy or LEEP performed within 1 year of enrollment, in response to a safety notification based on enrollment specimens, was considered part of this baseline study.

Statistical Analyses

The primary study endpoint case definition was established *a priori* as a Pathology Quality Control Group histologic diagnosis of CIN3+ (CIN3, adenocarcinoma *in situ,* or cancer). However, current clinical practice in the United States is based on treatment of histologically confirmed CIN2+ (CIN2 or CIN3+). Therefore, this endpoint is included as a secondary analysis.

The binomial distribution was used to compute exact confidence intervals (CIs) for proportions (e.g., sensitivity). Based on the similar findings in the HPV triage and immediate colposcopy arms (*see* the "Results" section), these data were combined for sensitivity determinations. Pearson's chi-square tests for contingency tables were used to assess the associations between categorical variables (e.g., cytologic diagnoses versus HPV test results). McNemar's test was used to assess the significance of differences in paired data, such as the comparison of the sensitivities of cytology and HPV testing in the same subjects. Chi-square statistics for trend were calculated to test the significance of data with evident ordering (such as increasing severity of cytologic diagnoses related to HPV positivity). All statistical tests were two-sided and were considered to be statistically significant at $P<.05$.

Results

Study Population

In total, 3488 eligible women referred with a community cytology diagnosis of ASCUS were enrolled in ALTS. Randomization yielded 1163 in the immediate colposcopy arm, 1161 in the HPV triage arm, and 1164 in the conservative management arm (Fig. 1). The mean age of participants was 29 years and was similar for all four study centers and for each arm *(2).*

The composition of the study populations varied by center. However, the randomization was stratified by center; therefore, the study arms were balanced with regard to race, ethnicity, and known behavioral risk factors for cervical cancer and squamous intraepithelial lesions, such as lifetime number of sexual partners, age at first intercourse, parity, years of education, and number of Pap smears in the preceding 5 years *(2).* There were no statistically significant differences between study arms for HPV results, clinical center enrollment cytology diagnoses, or Pathology Quality Control Group review of referral smears and enrollment cytology *(2).*

Pathology Quality Control Group Review of Index Referral Smear

The Pathology Quality Control Group reviewed 3389 (97%) of 3488 of the referral ASCUS smears that brought the women into the trial. In 55% of the cases, the Pathology Quality Control Group concurred with the diagnosis of ASCUS; 31% of the cases were downgraded to negative and 14% were upgraded to squamous intraepithelial lesions (11% LSILs and 3% HSILs).

HC 2 Results

Overall, the HC 2 test was positive in 1766 (50.6%) of the 3488 participants and ranged from 31.0% to 59.7% by clinical center. The HC 2 test results of 164 (4.7%) of the 3488 women were missing, most often because of an insufficient amount of residual specimen in the PreservCyt vial. Therefore, the overall triage to colposcopy, based on a positive or a missing HC 2 result, would be 55.3% (95% CI = 53.7% to 57.0%) if this triage strategy had been employed on *all* women in the trial.

Enrollment Cytology Results

PreservCyt specimens were collected from 3485 (99.9%) of the 3488 enrolled women. An additional specimen was obtained in fewer than 1% of cases because the first was unsatisfactory, most commonly as a result of scant squamous epithelial cellularity.

Table 1 shows the clinical center cytology results compared with the HPV test results. (All arms are combined for analysis purposes, although the HPV result was only unmasked and used for triage in the HPV arm.) Overall, at the centers, the majority of specimens were called negative (41.9%) or ASCUS (32.5%); 18.1% were LSILs and only 7.0% showed HSILs (range, 2.9%–11.3% by clinical center). The trend toward increasing HPV positivity with increasing severity of cytology diagnoses was significant ($P<.001$ for trend test). When we eliminated the specimens that were missing an HPV result (n = 164), 32.7% (95% CI = 30.2% to 35.2%) of the negative cytology results, 50.6% (95% CI = 47.6% to 53.6%) of ASCUS, 88.7% (95% CI = 85.8% to 91.1%) of LSIL, and 97.0% (95% CI = 93.9% to 98.8%) of HSIL cytologies were HPV positive. When we ex-

Table 1. Clinical Center cytology diagnoses by human papillomavirus (HPV) testing result: all study arms*

Clinical Center cytology	HPV results by HC 2† Negative (row %)	Missing (row %)	Positive (row %)	Total (column %)
Missing	9 (50.0)	3 (16.7)	6 (33.3)	18 (0.5)
Negative	934 (64.0)	73 (5.0)	453 (31.0)	1460 (41.9)
ASCUS	541 (47.7)	38 (3.4)	555 (48.9)	1134 (32.5)
LSIL	67 (10.6)	38 (6.0)	525 (83.3)	630 (18.1)
HSIL-CIN2	7 (3.4)	9 (4.3)	191 (92.3)	207 (5.9)
HSIL-CIN3+	0 (0)	3 (7.7)	36 (92.3)	39 (1.1)
Total	1558 (44.7)	164 (4.7)	1766 (50.6)	3488 (100)

*ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HSIL = high-grade squamous intraepithelial lesion; CIN2 = cervical intraepithelial neoplasia grade 2, CIN3+ = cervical intraepithelial neoplasia grade 3 or worse.

†Hybrid Capture 2™ includes probes for cancer-associated HPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

cluded unsatisfactory specimens, there was no statistically significant difference in cytology diagnoses in women with missing HPV results and those with HPV results.

Colposcopy Findings

Based on enrollment test results and the protocol algorithm, 100% of women in the immediate colposcopy arm, 56.1% (95% CI = 53.2% to 59.0%) in the HPV triage arm, and 8.7% (95% CI = 7.1% to 10.4%) in the conservative management arm were triaged to colposcopy. In the HPV triage arm, virtually no triage was based on HSIL cytology alone (n = 1). Six women (four in the HPV triage arm and two in the conservative management arm) were triaged to colposcopy because a worrisome lesion was seen on the enrollment pelvic examination. In addition, two women in the conservative management arm were triaged on the basis of a Pathology Quality Control Group safety net review of the ThinPrep enrollment cytology.

Some women triaged to colposcopy refused the procedure or were lost to follow-up. The percentage of women who failed to have an anticipated colposcopy varied by arm: 1.2% in the immediate colposcopy arm, 6.1% in the HPV triage arm, and 6.9% in the conservative management arm ($P<.001$). In other words, fewer women refused "immediate" colposcopy in the immediate colposcopy arm as compared with the loss to follow-up experienced with "recall" colposcopy for the HPV triage and conservative management arms. The median time from enrollment to colposcopy, for those who attended, was less than 1 day for immediate colposcopy, 56 days for HPV triage, and 36 days for conservative management.

Table 2 shows the distribution of the clinical center colposcopist's impression by study arm. Almost all of the women in the immediate colposcopy arm had colposcopy; therefore, most of these women had either negative (including cervicitis, atrophy, polyp, or atypical metaplasia) (41.0%) or CIN1 (51.4%) colposcopic findings: Only 7.0% of examinations were considered to be CIN2 or more severe. Compared with immediate colposcopy, HPV triage increased the percent of colposcopic findings that were CIN1 (59.2%) and CIN2 or greater (11.9%) ($P<.001$ for trend test). As expected on the basis of the triage criterion of HSIL+ cytology, a majority of the colposcopy examinations in the conservative management arm showed changes considered to be CIN2 or greater (57.4%); only 6.4%

Table 2. Colposcopy impression by study arm among those triaged to colposcopy*

Colposcopic impression	Study arm: Immediate colposcopy	HPV triage	Conservative management
Unsatisfactory or missing	7 (0.6%)	6 (1.0%)	3 (3.2%)
Negative	471 (41.0%)	170 (27.8%)	6 (6.4%)
CIN1	591 (51.4%)	362 (59.2%)	31 (33.0%)
CIN2 or greater	80 (7.0%)	73 (11.9%)	54 (57.4%)
Total†	1149 (100%)	611 (100%)	94 (100%)

*HPV = human papillomavirus; CIN1 = cervical intraepithelial neoplasia grade 1; CIN2 = cervical intraepithelial neoplasia grade 2.

†Limited to women who had colposcopy.

were considered to be negative by examination (trend test $P<.001$ for immediate colposcopy compared with conservative management and for HPV compared with conservative management). In contrast to the immediate colposcopy arm, in the HPV and conservative management arms, colposcopists were aware of the triage test results at the time of colposcopy, which may have influenced clinical assessment of the cervix.

Histology Results

Directed biopsy was performed if any CIN lesion was suspected by colposcopic examination. If the clinical center biopsy results showed CIN2+, then LEEP was performed.

Although clinical management was based on the clinical center diagnoses, Pathology Quality Control Group review diagnoses were used for final case definitions. In some cases, more severe disease was detected in the larger LEEP tissue specimen than at biopsy. The most severe histologic finding per woman was then considered to be her final enrollment histologic diagnosis.

Results obtained by the Pathology Quality Control Group review of histology are shown in Table 3. Looking by arm, the same trends as seen with colposcopic impression were apparent with tissue diagnoses. As a percentage of all histologic diagnoses, a shift to higher grade disease was seen in the HPV and conservative management arms as compared with the immediate colposcopy arm ($P<.001$ for trend test). As a percentage of all women enrolled (not just those who had tissue biopsy as shown in Table 3), more CIN1 was found in the immediate colposcopy

Table 3. Pathology Quality Control Group biopsy diagnoses by study arm*

Quality control histology	Study arm: Immediate colposcopy	Human papillomavirus triage	Conservative management
Negative	539 (62.9%)	237 (48.0%)	19 (20.4%)
Squamous atypia	20 (2.3%)	10 (2.0%)	2 (2.2%)
CIN1	167 (19.5%)	111 (22.5%)	16 (17.2%)
CIN2	72 (8.4%)	59 (11.9%)	12 (12.9%)
CIN3+	59 (6.9%)	77 (15.6%)†	44 (47.3%)†,‡
Total§	857 (100%)	494 (100%)	93 (100%)

*CIN = cervical intraepithelial neoplasia. CIN1 = CIN grade 1; CIN2 = CIN grade 2; CIN3+ = CIN grade 3 or worse.

†Includes one case of adenocarcinoma *in situ.*

‡Includes one case of squamous cell carcinoma.

§Limited to women who had loop electrosurgical excision procedure and/or biopsy.

arm (n = 167 of 1163) than in the HPV (n = 111 of 1161) or conservative management (n = 16 of 1164) arms. The proportion of cases of CIN2 and CIN3+ was comparable in the HPV and immediate colposcopy arms (i.e., 136 of 1161 and 131 of 1163, respectively; $P = .75$). The proportion of CIN2 and CIN3+ cases in the conservative management arm was significantly lower than the proportion of CIN2 and CIN3+ cases in the combined immediate colposcopy and HPV arms (i.e., n = 56 of 1164 and n = 267 of 2324, respectively; $P<.001$). A statistically significant difference between the conservative management arm and the combined immediate colposcopy and HPV arms was also seen for the proportion of CIN3+ cases only (i.e., n = 44 of 1164 and n = 136 of 2324, respectively; $P = .01$).

Prevalence of Disease

If we assume that a colposcopically directed biopsy provides virtually complete ascertainment of disease, then the immediate colposcopy arm reflects the distribution of disease in the ASCUS trial population at enrollment. Evaluation of 1149 women who underwent colposcopy in the immediate colposcopy arm yielded the following prevalence percentages based on the Pathology Quality Control Group histologic diagnoses: 25.4%—no lesion identified at colposcopy and no biopsy (therefore, not included in Table 3); 46.9%—no pathologic lesion on biopsy; 1.7%—atypical squamous changes; 14.5%—CIN1; 6.3%—CIN2; and 5.1%—CIN3+.

Test Sensitivity

Comparable numbers of cases of histologically confirmed CIN2+ and CIN3+ were detected in the HPV arm compared with the immediate colposcopy arm, suggesting complete capture of high-grade disease in the HPV arm. Therefore, for the following test sensitivity determinations, the immediate colposcopy and HPV arms were combined to achieve maximal statistical power.

Table 4 shows the HC 2 and clinical center cytology results (expressed in terms of triage threshold of <HSIL versus HSIL+) for the 136 cases of Pathology Quality Control Group histologically diagnosed CIN3+ detected in the immediate colposcopy and HPV triage arms. The HC 2 test for HPV was positive in 125 and missing in six women, resulting in a total triage sensitivity of 131 (96.3%) of 136 (95% CI = 91.6% to 98.8%) cases. Cytology was less sensitive; 60 (44.1%) of the 136 cases (95% CI = 35.6% to 52.9%) showed HSIL+ cytology, as diagnosed by the clinical center ($P<.001$, McNemar's paired test). Four of the five cases of CIN3+ with a negative HC 2 test result did not have a cytologic diagnosis of HSIL+; therefore, adding cytology did not meaningfully increase the triage sensitivity compared with HPV alone.

Table 4. Triage sensitivity of cytology and HPV for detection of CIN grade 3 or worse in the combined HPV triage and immediate colposcopy arms*

Clinical Center cytology	HPV results by HC 2			
	Negative	Missing	Positive	Total
<HSIL	4	1	71	76
HSIL+	1	5	54	60
Total	5	6	125	136

*HPV = human papillomavirus; CIN = cervical intraepithelial neoplasia; HSIL+ = high-grade squamous intraepithelial lesion or above; HC 2 = Hybrid Capture 2™.

Test sensitivity varies, depending on the threshold established for a positive test as well as on the definition of the disease to be detected. In the trial protocol, HSIL+ was used as the threshold for cytologic triage to colposcopy in the HPV triage and conservative management arms. The sensitivity for detection of histologic CIN2+ and CIN3+ at lower cytology thresholds was determined from the cross-sectional analysis of enrollment cytology results in the immediate colposcopy and HPV triage arms. Table 5 compares the triage sensitivity, the percentage of women who would be referred to colposcopy, the positive predictive value, and the negative predictive value for detection of two levels of disease (CIN3+ and CIN2+) with the use of the following test thresholds: HC 2 positive at 1 pg or cytology at ASCUS+, LSIL+, or HSIL+. (*Note* that Table 5 is based on the combined HPV triage and immediate colposcopy arms [n = 2324] and that the referral percentages in Table 5 differ from the actual referral percentages in the trial protocol that included triage based on visual appearance of the cervix as well as on safety nets.) With the use of HC 2, 56.1% (95% CI = 54.1% to 58.1%) of women would be referred to colposcopy compared with only 6.9% (95% CI = 5.9% to 8.0%) for HSIL+ cytology. For both levels of disease, cytology at a threshold of HSIL+ was much less sensitive than HC 2 but referred far fewer women to colposcopy and had a higher positive predictive value. At the lowest cytology threshold of ASCUS+, the sensitivity for CIN3+ improved to 85.3% (95% CI = 78.2% to 90.8%) but with 58.6% (95% CI = 56.5% to 60.6%) of women referred to colposcopy.

Discussion

Our results indicate the excellent sensitivity of a well-validated HPV assay, HC 2 testing, for the detection of cervical cancer precursor lesions. This sensitivity, combined with reasonable specificity for triage, makes HPV testing a viable option for the management of ASCUS.

The follow-up of the estimated 2–3 million women *(7)* with a cytologic diagnosis of ASCUS in the United States each year represents a substantial financial cost for the health care system

Table 5. Triage test performance of HC 2 and cytology at different thresholds for detection of histologically confirmed CIN3+ and CIN2+, in the combined human papillomavirus (HPV) triage and immediate colposcopy arms*

	% sensitivity	% referral†	Positive predictive value‡	Negative predictive value§
CIN3+				
HC 2‖	96.3	56.1	10.0	99.5
HSIL+ cytology	44.1	6.9	37.5	96.5
LSIL+ cytology	64.0	26.2	14.3	97.1
ASCUS+ cytology	85.3	58.6	8.5	97.9
CIN2+				
HC 2‖	95.9	56.1	19.6	98.9
HSIL+ cytology	34.8	6.9	58.1	92.0
LSIL+ cytology	59.2	26.2	25.9	93.6
ASCUS+ cytology	85.0	58.6	16.7	95.8

*HC 2 = Hybrid Capture 2™; CIN = cervical intraepithelial neoplasia; HSIL+ = high-grade squamous intraepithelial lesion or above; LSIL+ = low-grade squamous intraepithelial lesion or above; ASCUS+ = atypical squamous cells of undetermined significance or above.

†Percent of the study population that would have been referred to colposcopy, with the use of a particular triage test threshold.

‡For positive test results, the percent of time disease was present.

§For negative test results, the percent of time disease was absent.

‖HC 2 at a positive test threshold of 1.0 pg of HPV DNA/mL.

and a financial as well as an emotional burden for the women affected. A diagnosis of ASCUS identifies a woman who is at a greater than background risk for prevalent and incipient CIN2, CIN3, and cancer. Previous studies *(8–12)* have shown that 20%–60% of ASCUS changes are associated with CIN at colposcopic evaluation, but the vast majority of these (>70%) are CIN1, a sign of usually benign HPV infection. Therefore, a balance must be achieved between excessive evaluation of cytologic changes that, in the majority of cases, would regress spontaneously and failure to diagnosis the small minority of women at risk for a true cancer precursor.

ALTS is a large, randomized, multicenter trial designed to compare management strategies for women with ASCUS or LSIL cytology results. This article summarizes enrollment data for women referred with a community diagnosis of ASCUS. Of note, the enrollment cytology and HPV assay results were performed an average of 2 months after the index referral ASCUS smear. This interval of time may have allowed for regression or possibly progression of lesions that would affect triage test performance. Also, repeat cytology performed less than 3 months after the index cytology is thought to be associated with decreased sensitivity; however, no time interval effects on cytology sensitivity were noted (data not shown) (Johnson G, Solomon D: unpublished data). Use of a liquid-based collection method (which is considered to be more sensitive than conventional smears) may have resulted in an optimized estimate of the sensitivity of cytology in the trial.

Among participants referred with ASCUS who had adequate test results, the proportion with HPV positivity showed a much wider range by center (31.0%–59.7%) than was seen among women referred with LSIL cytology results (79.1%–86.1%) *(3).* This finding reflected greater community variability in the use of ASCUS as a cytology diagnosis compared with LSIL. Heterogeneity among participants by center was also seen in the percentage of HSILs diagnosed on the enrollment (repeat) cytology (2.9%–11.3%) among women referred with ASCUS index smears. Despite the distinct center differences, randomization was stratified by center; therefore, the study arms were balanced.

The ALTS design assumed *a priori* that the immediate colposcopy arm would result in complete ascertainment of disease endpoints and would provide a standard to which the HPV and the conservative management arms would be compared. The prevalence of histologically confirmed CIN2+ disease in the immediate colposcopy arm was in the range of previous U.S. studies of ASCUS patients *(8–11).* However, relatively less CIN1 was diagnosed, probably reflecting the Pathology Quality Control Group's more stringent threshold for this diagnosis compared with pathologists generally.

Referral of a little over half of the women for colposcopy in the HPV arm yielded comparable numbers of cases of tissue-confirmed CIN2+ as compared with immediate colposcopy. However, fewer cases of CIN1 were diagnosed. The HC 2 assay did not include low-risk HPV types; therefore, a proportion of CIN1 (associated with low-risk HPV types) was likely HPV test negative and hence not triaged to colposcopy. In addition, the median time to colposcopy in the immediate colposcopy arm was less than 1 day, but the HPV triage entailed a median 8-week delay before colposcopy, perhaps allowing for regression of some lesions in the interim. Therefore, the missed CIN1 in the HPV arm may represent a very low risk subgroup of CIN1 that was due to infection with low-risk HPV types or that regressed within months. This deficit may actually represent a reduction in unnecessary follow-up and patient anxiety for a subgroup of women with CIN1.

The triage sensitivity for the detection of the study endpoint of histologically diagnosed CIN3+ in the combined immediate colposcopy and HPV arms was 96.3% (95% CI = 91.6% to 98.8%) for HC 2 testing and 44.1% (95% CI = 35.6% to 52.9%) for cytology at the HSIL+ threshold. Several points must be emphasized regarding the sensitivity of cytology for detection of CIN3+ cases. First, the conventional management strategy of cytologic follow-up is based on a series of repeat cytology, not on the sensitivity of a single cytologic sampling. Therefore, the total program sensitivity of cytology must await longitudinal follow-up, with repeat cytology performed every 6 months. Second, in ALTS, a high cytologic referral threshold of HSIL was established in the hope of demonstrating sensitive detection of CIN3+ over time while avoiding excessive referral to colposcopy. At the lower cytologic referral threshold of ASCUS or above, sensitivity improved significantly (although not to the level of HPV DNA testing), with comparable numbers of women referred to colposcopy compared with HPV testing. Third, at this time, we do not know the clinical significance of cytologically occult CIN3. CIN3 lesions identified by colposcopy, in the absence of HSIL cytology, may not have the same biologic behavior as conventionally diagnosed CIN3. Topographically, cytologically occult CIN3 lesions tended, in fact, to be somewhat smaller than those associated with HSIL cytology, as crudely measured by the number of histology blocks involved by disease (data not shown). Longitudinal follow-up will address these questions.

The triage sensitivity of HC 2 for detection of histologically diagnosed CIN2+ in the immediate colposcopy and HPV arms was 95.9% (95% CI = 92.8% to 97.9%). Previous smaller U.S. studies using HC 2 as a triage strategy for ASCUS *(9–11)* have shown a slightly lower range of sensitivity for CIN2+ (78%–90%). In the study by Manos et al. *(10),* only 11 of 13 cancer-associated HPV types were included in the HC 2 assay; the other two studies *(11,12)* involved fewer than 300 women, and the sensitivity may have been slightly affected by a learning curve for HC 2 testing.

In the context of an ASCUS cytologic diagnosis, HC 2 testing for cancer-associated HPV types is a sensitive triage method for detection of CIN3+ and CIN2+. In this patient population, a little over half of the women would be referred to colposcopy on the basis of the HC 2 test alone. The addition of a single repeat cytology to the triage strategy (using a threshold of HSIL+ cytology) did not substantially increase the sensitivity for CIN3+. Additional analyses will examine whether the specificity of HPV testing can be improved without sacrificing sensitivity by raising the threshold for a positive result, by tailoring recommendations based on patient age, or by lengthening the time interval from index ASCUS cytology result to HPV testing (Sherman ME, Schiffman M, Cox JT: unpublished data).

APPENDIX

The affiliations of the ALTS (i.e., the Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study) Group are as follows:

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NOTES

Editor's note: J. T. Cox is on the speaker's bureau and participates occasionally in the medical advisory boards for the Cytyc Corporation (Boxborough, MA) and the Digene Corporation (Gaithersburg, MD). R. S. Guido does research for 3M (St. Paul, MN). A. Lorincz is Senior Vice President and Chief Scientific Officer of the Digene Corporation and holds stock and stock options in the company. M. M. Manos holds stock in the Cytyc Corporation and serves as an *ad hoc* consultant to the company. M. E. Sherman receives research support from the Digene Corporation, TriPath Imaging (Elon, NC), the Cytyc Corporation, and the National Testing Laboratories (Fenton, MO) and has a contract with Merck Corporation (West Point, PA).

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